

## **Description**

A Synthetic CpG-containing single-stranded oligodeoxynucleotide against positive single -stranded RNA viruses

## **FIELD OF THE INVENTION**

The present invention relates generally to the synthetic CpG-containing single-stranded oligodeoxynucleotides against positive single-stranded RNA viruses, and more particularly to CpG-containing single-stranded ODNs against severe acute respiratory syndrome coronavirus ( SARS-CoV ), hepatitis C virus, Dengue virus, and Japanese encephalitis virus. The invention also relates to the therapeutic and prophylactic effect of CpG-containing single-stranded ODNs on infectious diseases caused by positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus, as well as techniques and assumptions of treating and preventing infectious diseases caused by positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus using CpG-containing single-stranded ODNs.

## **BACKGROUND:**

The studies in recent years indicate that many different bacterial and viral DNAs containing CpG dinucleotide(s) are danger signals to the human

immune system. They can activate many kinds of immunocytes and induce immune response of the organism against viruses. “CpG” refers to the dinucleotide, where C is cytosine, G is guanine, and p represents the phosphodiester bond between the two. Further studies have showed that synthetic CpG-containing single-stranded ODNs can also activate immunocytes and induce immune response of the organism against viruses.

SARS-CoV, the pathogen that has caused severe acute respiratory syndrome (SARS) is a mutated coronavirus, which belongs to positive single-stranded RNA viruses with the viral envelops. ( Christian Drosten, Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome The New England Journal Of Medicine ,2003 ,348;19 ; Paul A. Rota, etal, Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome Science, Vol. 300, Issue 5624, 1394-1399, May 30, 2003 ) Hepatitis C virus, the pathogen that has caused hepatitis C is a positive single-stranded RNA virus with the viral envelop as well. ( Lindenbach, B. D., and C. M. Rice. 2001. Flaviviridae: the viruses and their replication, p. 991-1041. In D. M. Knipe and P. M. Howley (ed.), Fields virology, vol. 1. Lippincott/The Williams and Wilkins Co., Philadelphia, Pa ) Hepatitis C virus is a member of the Flaviviridae family. Another two members in the Flaviviridae family are Dengue virus ( Wei-Kung Wang, Su-Ru Lin, Chao-Min Lee, Chwan-Chuen King, and Shan-Chwen Chang Dengue Type 3 Virus in Plasma Is a Population of Closely Related Genomes:

Quasispecies Journal of Virology, May 2002, p. 4662-4665, Vol. 76, No. 9 ) and Japanese encephalitis virus (Sang-Im Yun, Seok-Yong Kim, Charles M. Rice, and Young-Min Lee Development and Application of a Reverse Genetics System for Japanese Encephalitis Virus Journal of Virology, June 2003, p. 6450-6465, Vol. 77, No. 11), which are also enveloped, positive-sense, single-stranded RNA viruses.

## CONTENT OF THE INVENTION

### Summary of the invention

The first purpose of the present invention is to provide the synthetic CpG-containing single-stranded ODN, especially the one that can stimulate human peripheral blood mononuclear cells (PBMCs) to produce the substances against single-stranded RNA viruses. The synthetic CpG-containing single-stranded ODN consists of single-stranded oligodeoxynucleotide containing one or more CpG motifs. The CpG-containing single-stranded ODN described herein can be partly or completely phosphorothioate-modified, or unmodified. The optimized CpG-containing single-stranded ODN sequence according to this invention are showed in SEQ ID NO : 1.

The second purpose of the invention is to illustrate the effect of the synthetic CpG-containing single-stranded ODN according to this invention on resisting positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus.

The third purpose of the invention is to illustrate the therapeutic and prophylactic effect of synthetic CpG-containing single-stranded ODN on infectious diseases caused by positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus.

The fourth purpose of the invention is to provide techniques and assumptions of treating and preventing infectious diseases caused by positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus using the synthetic CpG-containing single-stranded ODN of the invention.

In addition, it should be pointed out that based on Applicants' disclosure of the present invention, a variety of other uses that flow naturally from the instant disclosure and which would be readily apparent to one of skill the art.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

In the context of this invention, the terms are well known to those skilled in the art of this field, except for additional explanations. Specifically, the following terms have the meanings below :

The optimized single-stranded oligodeoxynucleotide containing CpG motifs according to the present invention has a sequence listed below:

SEQ ID NO : 1

DVAX-1 : 5'-TCgTCgggTgCgACgTCgCAggggggg -3'

The single-stranded oligodeoxynucleotide containing CpG motifs described herein can be partly or completely phosphorothioate-modified, or unmodified.

The artificial single-stranded oligodeoxynucleotide (ODN) containing CpG motifs of this invention can be synthesized by techniques well known in the art, such as solid phase phosphoramidite trimer method. The following embodiments illustrate exemplary methods of producing such compositions according to the invention in detail.

The compounds described herein can be used for the therapy and treatment of human infectious diseases caused by positive single-stranded RNA viruses, especially SARS-CoV, hepatitis C virus, Dengue virus, and Japanese encephalitis virus, with dosages between 1 µg and 5000 µg /dose.

The synthetic CpG-containing single-stranded ODN according to the present invention is used alone or in combination with other drugs or vaccines against positive single-stranded RNA viruses.

The composition described herein can be administered by gastrointestinally, in an ophthalmic preparation, by injection subcutaneously, intramuscularly, intravenously, intraperitoneally, and by mucosal application including the mucosa of respiratory tract, gastrointestinal tract and genitourinary tract.

Further detailed descriptions of the present invention are given by reference to the following examples of preparation and biological function in conjunction with the accompanying figures. It should be understood that these instances are for purposes of illustration only and are not intended to limit the invention by any manner of means.

In the embodiments below, various procedures and methods not given all details are well known to one skilled in the art. For instance, the method of solid phase phosphoramidite trimer is used for synthesizing the composition according to this invention. In the following examples, the source, trade name, and components which are necessary to list of the reagents are described for once only. There are not any repeated contents for the same reagent afterwards.

#### Example 1

Preparation of the Synthetic Single-Stranded Oligodeoxynucleotides Containing CpG motifs

Solid-phase 3'-phosphoramidites method was used for synthesis of the single-stranded oligodeoxynucleotides containing CpG motifs

#### 1. Reagents and Materials

Trichloroacetic Acid(TCA), Controlled Pore Glass ( CPG ) , DMT, Tetrazole activator, Acetic anhydride, N-methylimidazole, Deoxynucleoside

triphosphate(dNTP A, T, C, G), ABI DNA synthesizer, Sensitive Liquid Chromatograph, etc.

## **2. Methods**

### **Deblocking**

Deblocked off the DTM (a nucleotide blocking group) from controlled pore glass (CPG) by trichloroacetic acid(TCA) to obtain a free 5'-OH group for condensation reaction.

### **Activation**

Put the phosphoramidite protected nucleotide monomers and tetrazole activator mixture into composite column together to get a phosphoramidite tetrazolium reactive intermediate. This intermediate (activation in 3' terminal, DMT protection in 5' terminal) would be condensed with deblocked nucleotides on controlled pore glass (CPG).

### **Coupling**

When encountered with the deblocked nucleotides on controlled pore glass (CPG), phosphoramidite tetrazolium reactive intermediate would affinity with their 5'-OH and condensed to deblock off tetrazolium, then the synthetic oligonucleotides extended for one basic radical.

### **Capping**

To prevent the extension of nonreactated 5'-OH on controlled pore glass in the sequential circulations after condensation reaction, we often capped this 5'-OH by acetylation. Generally, the acetylation reagent was generated by

mixing acetic anhydride and N-methylimidazole.

#### Oxidation

Nucleotide monomers were connected with oligomers on the controlled pore glass by phosphite bonds in condensation reaction. But phosphite bonds were instable and prone to be hydrolyticed by acid and base, therefore we often transformed phosphoryl into phosphotriester by iodine tetrahydrofuran solution to obtain stable oligomers.

After the five procedures above, a deryonucleotide was linked to the nucleotides on controlled pore glass. Samely, deblocked off the DTM (a nucleotide blocking group) from the latest deryoneclotides 5'-OH by trichloroacetic acid and repeated the activation, coupling, capping and oxidation, we obtained a coarse DNA fragment. Finally, the coarse DNA fragment would be cutted, deblocked (benzoyl protection in A and C, isobutyryl protection in G, no protection in T, phosphorous acid is protected by nitrile ethyl), purified (such as HAP, PAGE, HPLC, C18, OPC etc) and quantified etc, then oligonucleotide fragments according to experimental requirements would be obtained.

Nonphosphorothioate-modified single-stranded oligodeoxynucleotides containing CpG motifs were synthesised on ABI 3900 DNA synthesizer; fully or partly phosphorothioate-modified single-stranded oligodeoxynucleotides containing CpG motifs were synthesised on ABI 394 DNA synthesizer by displacement method.



## **Example 2**

**Effect of the synthetic single-stranded oligodeoxynucleotide containing CpG motifs against SARS-CoV (a kind of positive single-stranded RNA viruses)**

### **1. Isolation of human PBMCs**

#### **(1) Materials:**

Heparinized human whole blood was provided by the Blood Center of Jilin Province, Changchun, China.

**Ficoll-Hypaque:** the specific weight was  $1.077 \pm 0.001$  (Beijing Dingguo Biotechnology Co., Ltd.).

**Cell culture instruments:** deep freeze refrigerator, CO<sub>2</sub> incubator, superclean bench, inverted microscope, nitrogen canister, distillator, vacuum pump, cell culture bottle, bacterial filter, filtered bottle, haustorial tubes with different ranges, sample injectors, droppers, blood cell counting plate and horizontal centrifuge.

#### **RPMI1640 culture medium:**

RPMI1640 powder containing

L-glutamine (GIBCOBRL)

10.4 g

NaHCO <sub>3</sub>	2.0 g
Gentamicin	100,000 unit

Tri-distilled water was added to the final volume of 1000mL. The medium was filtered using 0.22  $\mu$ m filter and vacuum pump and stored in small volumes.

**Inactivation of fetal calf serum (FCS):** The FCS (Invitrogen) was heat-inactivated for 30 min at 56°C, after being taken from deep freeze refrigerator (-20 °C) and thawed at 4 °C.

**RPMI1640 supplemented with 10% FCS:**

Inactivated FCS	10 ml
RPMI1640 culture medium	90 ml

**Hank's Solution (free of Mg<sup>2+</sup> and Ca<sup>2+</sup>):**

NaCl	8.0 g
KCl	0.4 g
Na <sub>2</sub> HPO <sub>4</sub> ·H <sub>2</sub> O	0.06 g
KH <sub>2</sub> PO <sub>4</sub>	0.06 g
NaHCO <sub>3</sub>	0.35 g
Glucose	1.0 g

Phenol red

0.02 g

Double distilled water was added up to the final volume of 1000 ml.

All the reagents were fully dissolved and mixed well, autoclaved for 15 min at 8 PSIA (pound per square inch) and stored at 4°C. Before used, the pH of the solution was adjusted to 7.3~7.6 with 7.4% NaHCO<sub>3</sub>.

**2% Trypan blue solution:**

Trypan blue

2.0 g

Physiological saline

100 ml

**(2) Methods:**

The heparinized blood was slowly overlaid the Ficoll-Hypaque solution (specific weight: 1.077±0.001) following the tubal wall, and the ratio of the volume of Ficoll-Hypaque to blood was 2:1.

The tube was centrifuged 15~20 min in a horizontal centrifuge at 1000 × g, and the liquid was demixed into three layers. The mononuclear cell layer in white was transferred to a new tube with sterile pipet.

The same volume of Hank's solution (free of serum) was added to the tube, mixed well and centrifuged for 15 min at 800~1000 ×g. The supernatant was discarded and this washing process was repeated twice again.

The mononuclear cells were resuspended in 2 ml RPMI1640 complete medium supplemented by 10% FCS.

A drop of cell suspension was diluted for cell counting. Total cell number of the four squares was counted and the density of the PBMC (cell number/ml) = (total cell numbers $\times 10^4$ ) /4.

## **2. Harvesting the supernatants of human PBMCs stimulated by CpG ODN ( DVAX-1 )**

2 ml/well of human PBMCs ( $4\times 10^6$ /ml) in complete RPMI1640 containing 10% FCS were cultured in 12-well plate with sterile CpG ODN (DVAX-1), the final concentrations of which were 25  $\mu$ g/ml, 12.5  $\mu$ g/ml, 6.25  $\mu$ g/ml and 3.13  $\mu$ g/ml. And the medium-control well was not added CpG ODN. After incubated for 48 h in a 5% CO<sub>2</sub> incubator at 37 °C, the supernatants were collected and stored at -20 °C.

## **3. Anti-SARS-CoV experiment:**

The experiment was accomplished in the Institute of Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

### **(1) Materials:**

VERO E6 cells were purchased from the Institute of Viral Disease Control and Prevention, Chinese Center for Disease Control and

## Prevention.

**SARS-CoV** (the Sino-5 stain, which was isolated from the No.4 acute-serum sample of SARS patients) was provided by Beijing YouAnMen Hospital that was identified and stored by the Institute of Virus Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

**Positive-control drug:** YuanCeSu (hrIFN $\alpha$ -2b) was purchased from BeiJing YuanCe Pharmaceutical Co., Ltd. (specifications: 1,000,000 IU/tube; authorized number: GYZZ S19990013; and batch number: 000503A).

## (2) Methods

The Vero E6 cells were seeded into 96-well plates ( $4 \times 10^5$  cells/ml, 200  $\mu$ l/well) and cultured at 37 °C in a 5% CO<sub>2</sub> humidified incubator for 24 hours. Then the cell culture supernatants were discarded and the cells were incubated continuously with 100 TCCID<sub>50</sub> of coronavirus (Sino-5 strain) for 2 hours. Then the supernatants containing the virus were discarded, and the cells were incubated continuously with 100  $\mu$ l of the diluted cell culture supernatants of CpG ODN (DVAX-1)-stimulated human PBMCs. Each dilution of the supernatants, hrIFN $\alpha$ -2b control, cell control and virus control were all evaluated in biplicates. After incubated for 6 days, the cytopathic effect (CPE) was observed with microscope. The cytopathic cells account for 25% of the total per well were

represented with “+”; 26%~50%, “++”; 51%~75%, “+++”; and 76%~100%, “++++”. After observation , 100 µl of 0.5% crystal violet (crystal violet 0.5 g and NaCl 0.85 g were dissolved in 50 ml absolute ether, 3ml formaldehyde and 47ml distilled water ) was added to each well and cultured for 15 minutes at 37°C in the 5%CO<sub>2</sub> incubator. And then the wells were washing with lotic water following added 100µl of destaining solution (ethylene alcohol mon-methyl ether 50ml added to distilled water 50ml) and vibrated at room temperature for 2 hours. The The absorbance was determined with an ELISA reader at 492 nm.

### (3) Results

#### ☐ Results of CPE method

The supernatants of human PBMCs stimulated with 25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 160 folds could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs stimulated with 12.5 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs stimulated with 6.25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 40 folds could inhibit coronaviruses (Sino-5 strain).

HrIFN $\alpha$ -2b diluted to more than 50, 000 IU could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs without CpG ODN-stimulation could not inhibit coronaviruses (Sino-5 strain).

□ Results of crystal violet staining method

The supernatants of human PBMCs stimulated with 25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs stimulated with 12.5 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs stimulated with 6.25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 20 folds could inhibit coronaviruses (Sino-5 strain).

HrIFN $\alpha$ -2b diluted to more than 50,000 IU could inhibit coronaviruses (Sino-5 strain).

The supernatants of human PBMCs without CpG ODN-stimulation could not inhibit coronaviruses (Sino-5 strain).

These results illustrate that the supernatants of CpG ODN (DVAX-1)-stimulated human PBMCs contain the substances that inhibit positive single-stranded RNA virus, SARS-CoV, and CpG ODN (DVAX-1) can exert anti-positive single-stranded RNA virus, SARS-CoV, activity by inducing cells to produce antiviral substances.

**Example 3:**

Effect of the synthetic single-stranded oligodeoxynucleotide containing CpG motifs against Dengue virus (a kind of positive single-stranded RNA viruses)

1. Isolation of human PBMCs

The procedure was the same as Example 2.

2. Collecting culture supernatants of human PBMCs stimulated by CpG ODN (DVAX-1)

The procedure was the same as Example 2.

3. Anti-Dengue virus experiment

( 1 ) Materials

Dengue virus (D2V strain NGC) was cultured in C6/36 insect cells (purchased from ATCC)

Vero cells were purchased from ATCC.

RPMI1640 complete culture medium containing 10% FCS was the same to example 2.

(2) Methods

The Vero E6 cells were seeded into 96-well plates ( $4 \times 10^5$  cells/ml, 200  $\mu$ l/well) and cultured at 37 °C in a 5% CO<sub>2</sub> humidified incubator for 24 hours. Then the cell culture supernatants were discarded and the cells were incubated continuously with 100 TCCID<sub>50</sub> of Dengue virus for 2 hours. Then the supernatants containing the virus were discarded, and the cells were incubated continuously with 100  $\mu$ l of the diluted cell culture



supernatants of CpG ODN (DVAX-1)-stimulated human PBMCs. Each dilution of the supernatants, cell control and virus control were all evaluated in triplicates. After incubated for 8 days, the cytopathic effect (CPE) was observed with microscope. The cytopathic cells account for 25% of the total per well were represented with “+”; 26%~50%, “++”; 51%~75%, “+++”; and 76%~100%, “++++”.

### (3) Results (observation of CPE)

The supernatants of human PBMCs stimulated with 25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit Dengue virus.

The supernatants of human PBMCs stimulated with 12.5 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit Dengue virus.

The supernatants of human PBMCs stimulated with 6.25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 40 folds could inhibit Dengue virus.

The supernatants of human PBMCs without CpG ODN-stimulation could not inhibit Dengue virus. And the cells of virus control were fully cytopathic.

These results illustrate that the supernatants of CpG ODN (DVAX-1)-stimulated human PBMCs contain the substances that inhibit positive single-stranded RNA virus, Dengue virus, and CpG

ODN (DVAX-1) can exerts anti-positive single-stranded RNA virus, Dengue virus, activity by inducing cells to produce antiviral substances.

#### Example 4

Effect of the synthetic single-stranded oligodeoxynucleotide containing CpG motifs against Japanese encephalitis virus (a kind of positive single-stranded RNA viruses)

##### 1. Isolation of human PBMCs

The procedure was the same as Example 2.

##### 2. Collection of the cell culture supernatants of CpG ODN (DVAX-1)-stimulated PBMCs.

The procedure was the same as Example 2.

##### 3. Anti-Japanese encephalitis virus assay

###### (1) Materials

Japanese encephalitis virus: from Changchun Institute of Biological Products.

BHK-21 cells: from Neimenggu Biopharmaceutical Company.

10% FCS RPMI1640 completed culture medium: the same to Example 2.

###### (2) Methods

The BHK-21 cells were seeded into 96-well plates ( $4 \times 10^5$  cells/ml, 200  $\mu$ l/well) and cultured at 37 °C in a 5% CO<sub>2</sub> humidified incubator for 24

hours. Then cell culture supernatants were discarded and the cells were incubated continuously with 100 TCID<sub>50</sub> of Japanese encephalitis virus for 2 hours. Then the supernatants containing the virus were discarded, and the cells were incubated continuously with 100 µl of the diluted cell culture supernatants of CpG ODN (DVAX-1)-stimulated human PBMCs. Each dilution of the supernatants, cell control and virus control were evaluated in triplicates. After incubated for 4 days, the cytopathic effect (CPE) was observed with microscope. The cytopathic cells account for 25% of the total per well were represented with “+”; 26%~50%, “++”; 51%~75%, “+++”; and 76%~100%, “++++”.

### (3) Results (observation of CPE)

The supernatants of human PBMCs stimulated with 25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 160 folds could inhibit Japanese encephalitis virus.

The supernatants of human PBMCs stimulated with 12.5 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 80 folds could inhibit Japanese encephalitis virus.

The supernatants of human PBMCs stimulated with 6.25 µg/ml CpG ODN (DVAX-1) for 48 hours diluted to 40 folds could inhibit Japanese encephalitis virus.

The supernatants of human PBMCs without CpG ODN-stimulation could not inhibit Japanese encephalitis virus. And the cells of virus

control were fully cytopathic.

These results illustrate that the supernatants of CpG ODN (DVAX-1)-stimulating human PBMCs contain the substances that inhibit positive single-stranded RNA viruses, Japanese encephalitis virus, and CpG ODN (DVAX-1) can exert anti-positive single-stranded RNA viruses, Japanese encephalitis virus, activities by inducing cells to produce antiviral substances.

Sequences

<110> Changchun Huapu Biotech Co., Ltd

<120> Synthetic single-stranded oligodeoxynucleotide containing unmethylated CpG dinucleotides with anti-positive single-stranded RNA virus activity

<160> 107

<170> Patent In version 3.1<210> 1

<211> 26

<212> DNA

<213> Artificial

<400> 1

tcgtcgggtg cgacgtcgca ggggg g

26

Claims:

1. Synthesized single-stranded oligodeoxynucleotide containing CpG motifs.
2. The oligodeoxynucleotide of claim 1, wherein the phosphodiester bonds can be partly or completely phosphorothioate-modified, or unmodified.
3. The oligodeoxynucleotide of claim 1, 2 comprises a sequence of sequence No.1.
4. The oligonucleotide of claim 1,2,3 induces cell to secrete materials against infection caused by positive single-stranded RNA virus.
5. The RNA virus of claim 4 is a mutant of coronavirus.
6. The mutant of coronavirus of claim 5 is SARS virus.
7. The RNA virus of claim 4 is a kind of flaviviridae virus.
8. The flaviviridae of Claim 7 is Hepatitis C virus.
9. The flaviviridae of Claim 7 is Dengue fever virus.
10. The flaviviridae of Claim 7 is Japanese encephalitis virus.
11. The oligodeoxynucleotide of claim 1, 2, 3, 4 is a mixture containing other agents.
12. The mixture of claim 11 contains drugs or/and vaccines against positive single-stranded RNA virus.
13. The oligodeoxynucleotide of claim 1, 2, 3,4 or the mixture of claim 11, 12 induces cell to secrete materials against positive single-stranded RNA viruses.
14. The oligodeoxynucleotide of claim 1, 2, 3,4 or the mixture of claim 11, 12 induces cell to secrete materials against positive single-stranded RNA virus in vitro.
15. The oligodeoxynucleotide of claim 1, 2, 3,4 or the mixture of claim 11, 12 can be used for preparation of drugs against positive single-stranded RNA virus.
16. The RNA virus of claim 12, 13, 14, 15 is a mutant of coronavirus.
17. The mutant of coronavirus of claim 16 is SARS virus.
18. The RNA virus of claim 12, 13, 14, 15 is a kind of flaviviridae virus.
19. The flaviviridae of Claim 18 is Hepatitis C virus.
20. The flaviviridae of Claim 18 is Dengue fever virus.
21. The flaviviridae of Claim 18 is Japanese encephalitis virus.

- 22.The method for sythesizing the oligonucleotide of claim 1, 2, 3,4.
- 23.The method of claim 22 is artificial synthesis method.
- 24.The method of claim 23 is Solid-phase 3'-phosphoramidites method.
- 25.The oligodeoxynucleotide of claim 1,2,3 induces cell to secrete product.
- 26.The product of claim 25 is in supernatant of culture medium.
- 27.The product of claim 25, 26 induces cell to secrete materials against infection caused by positive single-stranded RNA virus.
- 28.The RNA virus of claim 27 is a mutant of coronavirus.
- 29.The RNA virus of claim 28 is SARS virus.
- 30.The RNA virus of claim 27 is a kind of flaviviridae virus.
- 31.The flaviviridae of Claim 30 is Hepatitis C virus.
- 32.The flaviviridae of Claim 30 is Dengue fever virus.
- 33.The flaviviridae of Claim 30 is Japanese encephalitis virus.
- 34.The mixture can be used for treating infection caused by RNA virus of claim 27,28,29,30,31,32,33.
- 35.The mixture of claim 34 includes vaccines or drugs against positive single-stranded RNA virus.
- 36.The product of claim 25,26 or mixture of claim 34, 35 induces cell to secrete materials against infection of positive single-stranded RNA virus.
- 37.The product of claim 25,26 or mixture of claim 34, 35 induces cell to secrete materials against infection of positive single-stranded RNA virus in vitro.
- 38.The product of claim 25, 26 or mixture of claim 34, 35 can be a drug for preventing or treating infection caused by positive single-stranded RNA virus.
39. The virus of claim 36, 37, 38 is a mutant of coronavirus.
40. The virus of claim 39 is SARS virus.
- 41.The virus of claim 36, 37, 38 is a kind of flaviviridae virus.
- 42.The flaviviridae of claim 41 is Hepatitis C virus.
- 43.The flaviviridae of Claim 41 is Dengue fever virus.
- 44.The flaviviridae of Claim 41 is Japanese encephalitis virus.
- 45.The method for manufacturing the materials of claim 25, 26, 27, includes methods of culturing PBMC and stimulating PBMC with oligonucleotide of claim 1, 2, 3,4

### Abstract

Artificial CpG-containing single-stranded oligodeoxynucleotides against positive single-stranded RNA viruses

The invention provides artificial CpG-containing single-stranded oligodeoxynucleotides (ODNs), wherein the ODNs can stimulate human peripheral blood mononuclear cell (PBMC) to produce antiviral substances which can protect cells from damaging of positive single-stranded RNA viruses infection. The CpG containing single-stranded oligodeoxynucleotides can prevent or treat infectious diseases caused by positive single-stranded RNA viruses, such as SARS virus, hepatitis C virus, engue virus and Japanese encephalitis virus.